APPENDIX G

Final Report

Screening Study to Determine Lead Uptake Capacity of Selected Cultivars of Brown Mustard (*Brassica juncea*), Oriental Mustard (*Brassica juncea*), White Mustard (*Brassica hirta*), and Safflower (*Carthamus tinctorius*)

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Introduction

ER&S personnel were funded by the US Army Environmental Center during 1996 and 1997 to conduct greenhouse treatability and optimization studies for phytoremediation of lead-contaminated soil. This is an *in situ* method which uses plants, in conjunction with certain soil amendments, to extract lead from contaminated soils. In this approach, the soil amendments (acetic acid and the chelate EDTA) solubilize soil lead into a form that is available to the plant. Acidifying the soil causes dissolution of lead from the solid phases in the soil into the liquid phase (i.e., the soil solution). EDTA then complexes with the soluble lead and prevents it from re-precipitating in the soil into a form that is unavailable to plants. Although soil acidification alone or the use of EDTA without soil acidification will convert some of the soil lead into a plant-available form, the synergistic relationship between the two amendments usually produces the best results. The solubilized lead is taken up into the plant biomass which is harvested and removed from the contaminated area.

The plant species tested in the 1996 - 1997 treatability greenhouse studies were alfalfa, corn, sorghum-sudangrass, sunflower, Indian mustard, and white mustard. These studies showed corn to be an efficient warm season species for lead accumulation when a soil acidifier and a chelate were used to solubilize soil lead. White mustard appeared to be the most efficient cool season plant since it accumulated high concentrations of lead without the need for soil acidification, a step required for the other species tested. The results from these studies led to funding by the Environmental Security Technology Certification Program (ESTCP) of a two-year field demonstration in 1998, "Phytoremediation of Lead-Contaminated Soil at the Twin Cities Army Ammunition Plant (TCAAP)."

The 1998 field results at TCAAP (as measured by lead uptake in the crop) using corn as the warm season remediation species were entirely satisfactory. However, adverse environmental and field conditions later in the year resulted in marginal performance by the cool season white mustard crop, and lead uptake from the soil was below target levels. Excessive rainfall during the growing season resulted in a limited and shallow root system, and other contaminants in the soil, e.g., thallium and beryllium, may also have hampered root growth. This led to a search for a more extensively- and deeper-rooted variety of cool season crop that could perform well in TCAAP soil for use in the 1999 demonstration.

Discussions with commercial plant breeders, growers, and seed producers indicated that other crops in the same family as white mustard, such as the brown and oriental mustards, develop a more extensive rooting system. These also produce a larger biomass than white mustard, which would be desirable for a phytoremediation crop. A larger biomass generally equates to more water uptake, and thus the capacity for uptake of larger quantities of water-soluble metals. Although safflower is typically grown as a warm-season seed crop, it may also be grown as a cool-season forage crop by delaying planting until midsummer. This plant species develops a deep rooting system, has a high transpiration rate conducive to extraction of water-soluble lead, and can produce a large forage biomass when grown as a cool season crop.

Objective

The objective of this study was to conduct a short-term plant screening study to determine the potential of brown mustard, oriental mustard, and safflower as alternative cool season phytoextraction crops to white mustard for lead removal in TCAAP soil. Specific objectives were to determine: (1) the lead uptake capacity of the plants; (2) the growth habit; (3) the need for soil acidification to optimize lead uptake; and (4) tolerance to adverse conditions in a soil such as that at TCAAP.

Materials and Methods

The plants were grown in soil from the Site C demonstration area at TCAAP which had been amended with acetic acid and EDTA as part of the 1998 field study (Table 1). This soil had a total lead content of 3,400 mg lead/kg soil. The amount of lead that would normally be considered as immediately plant-available, i.e., the water-soluble fraction, was negligible at a concentration of 12 mg/kg. Brown mustard (*Brassica juncea)*, oriental mustard (*Brassica juncea*), white mustard (*Brassica hirta)*, and two cultivars of safflower (*Carthamus tinctorius)* were grown from seed in 6-inch diameter, 7-inch deep plastic pots containing 1.65 kg of soil. Three replicates per treatment of soil-applied EDTA alone or EDTA plus acetic acid (HOAc) were used for each of the 5 species for a total 30 pots. No untreated controls were utilized, since previous greenhouse tests showed that lead uptake from such soil would be minimal compared to treated soils.

During the planting process, each crop received one-half of the optimum amount of nitrogen (N) fertilizer needed to satisfy the plant requirements for N, and all of the required potassium (K) fertilizer. Urea was used as the N source for the mustards at a rate of 260 pounds of N per acre, and ammonium nitrate was used for safflower at a rate of 115 pounds of N per acre. Phosphorus was supplied as concentrated super phosphate (CSP) at a rate of 100 pounds of P per acre for mustard and at 35 pounds of P per acre for safflower. Potassium sulfate was the K source at a rate of 130 pounds of K per acre for mustard and 100 pounds of K per acre for safflower. The second half of the N fertilizer was applied at 4 weeks growth for mustard and at 5 weeks for safflower.

Cool season crop environmental conditions were simulated in an air-conditioned TVA laboratory with artificial lighting (Environmental Growth Chamber Co.(EGC) high-pressure sodium, metal halide mix) under a 12-hour day length and an ambient temperature of 21^{o}C. The moisture content of the soil was maintained at field capacity (12%) throughout the growing period. However, safflower exhibited depressed early growth which may have been due to the cooler conditions in the laboratory, and at the end of the third week the plants were placed in the TVA

Muscle Shoals Research Greenhouse to in an attempt overcome any growth limitations imposed by the cool season conditions.

The soil acidifier (acetic acid) and the EDTA chelate were added to the mustard plants after the fifth week of growth, and to safflower after 7 weeks of growth. This was done by allowing the soil in the pots to dry to approximately two-thirds field capacity, then adding acetic acid to designated pots to reduce the soil pH to 5.5. The amount of acetic acid added was based on buffer curves previously determined on the TCAAP soil. The acidifier was followed by EDTA at a concentration equal to the molar concentration of lead in the soil. The amendments were added in a volume sufficient to return the soil to field capacity. This amount of solution ensured that the soil was wetted throughout the pot for maximum exposure of the plant roots to solubilized lead.

The mustard plants were harvested 48 hours after the amendment application; this time period had been shown in previous experiments to be adequate for maximum lead uptake to occur while preventing excessive drying and shattering of the plant tissue. Safflower was harvested 72 hours after the application when the plants were dessicated, but not so brittle as to shatter when handled.

The plant tissue was further dried in an oven at 65^{o}C, then ground in a Wiley mill equipped with stainless steel knives and screen. Following digestion, the tissue was then analyzed for total lead concentration by Inductively Coupled Argon Plasma (ICP) spectrometry. The data were analyzed statistically using ANOVA (analysis of variance) to separate treatment effects within species and among varieties. ANOVA is part of a software package from Statistical Analysis Systems (SAS) Institute, Cary, NC, for statistical analysis of variance in data.

Results and Discussion

The TCAAP soil used in this experiment is considered agronomically poor having a low nutrient content, a low cation exchange capacity, low organic matter content, low water-holding capacity, and high pH (Table 1). A low level of plant-available phosphorus (P) in the soil is the primary limiting factor for good plant growth. Normally, low P levels can be corrected with additional phosphate fertilizer. However, with phytoextraction schemes, this must be done with caution since supplemental P can complex soil Pb into insoluble forms and complicate Pb removal by the plant. Although the amount of P added at planting of mustard was fairly high, due to the short-term nature of this study this amount of P would not likely react with soil lead to significantly reduce lead availability to the crop. In a longer-term field situation, P applications would have to be judiciously applied to balance crop needs against the potential for excess lead complexation by P. Since this soil did not produce optimum growth of field crops during the 1998 demonstration season, N and K were over-supplied by 10% to encourage adequate growth of the crops.

Regardless of the increased initial amount of N-P-K fertilizer, or the additional N added during the growing period, all the plants exhibited a general lack of vigor and growth throughout the experiment. Stunting reduced expected growth rates of all plants by about one-third to one-half, depending on the species. Bolting of the mustard began at 4 weeks growth, instead of at the 6 to 8 week stage of growth that is typically observed. Safflower began flowering at 6 weeks, which is also atypical for this plant. The reduced growth and early bolting and flowering was most likely due to a combination of the overall poor quality of the soil and perhaps another contaminant in the soil, such as thallium (see Lehn and Schoer, 1987, Section 5.2.2.1) which was toxic to the plants. This pattern of reduced growth also occurred in the field for the white mustard crop at TCAAP in fall, 1998. Analysis of soil samples taken during the early growth of that crop appeared to rule out carry-over EDTA, soluble lead, or other metals as causative factors, but thallium was found at concentrations sufficiently high to be considered toxic. Safflower planted in an uncontaminated Lakeland sand for comparison under a similar fertility regime soil grew normally. However, untreated TCAAP soil was not used in this study.

In a separate study, the variety of brown mustard used herein exhibited very good growth on lead-contaminated soil obtained from the Volunteer Army Ammunition Plant (VAAP). The TCAAP soil and the VAAP soil were similar in texture and pH, and the two experiments have been conducted under almost identical fertility regimes. Several other metal contaminants which could potentially be toxic to plants, e.g., manganese, selenium, and zinc, were common to both soils. However, thallium was not a contaminant in the VAAP soil, and this could account for the difference in plant growth between the two soils.

The lead uptake capacity was essentially the same among the three mustard varieties if the soil was amended with EDTA without acidifying the soil (Table 2). However, lead concentrations in brown and oriental mustard plants doubled when EDTA was used in conjunction with acetic acid; this effect was not seen in white mustard. A similar pattern for lead uptake in white mustard was observed in previous greenhouse experiments conducted by ER&S researchers at Muscle Shoals in 1996-1997 ("Results of a Greenhouse Study Investigating the Phytoextraction of Lead from contaminated Soils Obtained from the Sunflower Army Ammunition Plant, Desoto, Kansas").

Lead concentrations in all mustard varieties were five- to tenfold lower than had been expected compared to results from the SFAAP experiments. Although the soils in the two studies were of similar pH and lead content, the SFAAP soil was very fertile, and plant growth was considerably better on that soil. The poor growth and early maturity caused by the adverse growing environment in the TCAAP soil most likely resulted in the reduced plant lead concentrations seen in this study.

Lead in the SFAAP soil was in a form that was amenable to complexation by the chelate and subsequent uptake by the plant. The chemical form of lead in soil (e.g., water-soluble, exchangeable, carbonate-bound, oxide-bound, organically-bound, and crystalline) controls the amount of lead complexation by EDTA. The water-soluble, carbonate- and oxide-bound forms, in that order, are more easily complexed by EDTA and potentially are the more plant-available forms. Due to the alkaline pH, a significant portion (>30%) of lead in the SFAAP soil was associated with the carbonate fraction. This form would be subject to ready dissolution by acetic acid and EDTA, which would make the lead available to the plant. However, in the highly buffered Sunflower soil, sequential extraction procedures showed that the overall equilibrium of lead among the various fractions remained relatively unchanged after an addition of acetic acid and EDTA, even though some lead was removed from the carbonate pool by the plant.

The various fractions of lead in the TCAAP soil have not yet been determined, but given the alkaline pH of the TCAAP soil, it would be logical to expect a significant portion of the soil lead to initially be present in the carbonate fraction. However, amendment additions and plant uptake of carbonate-bound lead in 1998 may have reduced the carbonate pool somewhat. Work is now in progress to determine the primary chemical forms of lead in the TCAAP soil.

In soil amended with EDTA alone, lead concentrations in safflower plants were about 50% lower than in mustard (Table 2). Acidifying the soil before adding EDTA resulted in lead concentrations in safflower statistically equivalent to the concentrations achieved in mustard without soil acidification. As with mustard, the overall poor growth of the plants, and the early flowering and termination of vegetative growth likely reduced the amount of lead taken into the plant. No information was available from the literature to indicate the levels of lead that might be expected in safflower. Therefore, the lead concentrations attained may be the limit for this species, and regardless of its other desirable qualities, safflower may not be suitable as a phytoextraction species for lead. However, safflower may have potential for use as an extraction crop for other metals.

Conclusions

Based solely on the lead concentrations found in the test plants, none of the five species would appear suitable for use as a phytoextraction crop for TCAAP soils. Of the plant species tested, the brown mustard, used in conjunction with soil acidification and EDTA, was the most effective at removing lead from the contaminated soil. Actual lead concentrations in the brown mustard under this treatment regime were about 7% greater than in Oriental mustard, although this difference was not statistically significant. A more definitive conclusion might be attained by growing the brown mustard under less adverse conditions, such as in another lead-contaminated soil of high fertility but which lacks plant-toxic constituents. Although safflower did not appear suitable for remediation of lead, the deep rooting system, high transpiration rate, and large biomass characteristics of the plant suggest that it may have potential for use with other metals.

Table 1
Partial Characterization of Pb-Contaminated Soil from Site C at TCAAP

Texture	**Sandy Loam**
pH	8.2
Cation exchange capacity, cmol/kg	4.9
Field capacity, %	12
Organic carbon, %	0.6
Total nitrogen, %	0.008
Exchangeable Ca, mg/kg	1,447
" Mg "	88
Extractable P, mg/kg	16
" K "	51
" Fe "	21
Total Pb, mg/kg	3,400
Plant available Pb, mg/kg	12

Table 2
Effect of Soil Amendments (EDTA Alone or EDTA Plus Acetic Acid - HOAc) on Lead Concentrations in Mustard and Safflower Plants

Plant	Treatment	Pb conc. in plant, mg/kg	
		Mean	s[1]
B. juncea - Brown mustard	EDTA	2,070	456
	EDTA + HOAc	4,257	653
B. juncea - Oriental mustard	EDTA	1,740	687
	EDTA + HOAc	3,990	567
B. hirta - White mustard	EDTA	2,327	133
	EDTA + HOAc	2,427	428
C. tinctorius - Safflower *cv* 1	EDTA	902	305
	EDTA + HOAc	2,497	442
C. tinctorius - Safflower *cv* 2	EDTA	1,125	663
	EDTA + HOAc	2,657	250
LSD (0.05) [2]		834	

[1] s - Standard deviation of the mean for 3 replicates of each treatment.

[2] Least Significant Difference at the 5% level of significance. ANOVA based on differences in Pb concentration in plants due to species and amendment effects.